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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Tyrphostins for Treatment of Allergic, Inflammatory and Cardiovascular Diseases

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(73) University of British Columbia (The) - Canada ;

(57) 10 Claims

Notice: The specification contained here: : as filed

TYRPHOSTINS FOR TREATMENT OF ALLERGIC, INFLAMMATORY AND CARDIOVASCULAR DISEASES

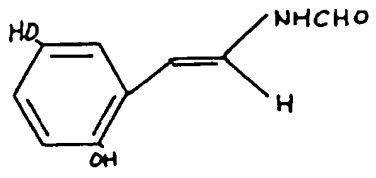
FIELD OF THE INVENTION

This invention pertains to the novel use of compositions containing benzylidene malononitrile and hydroxycinnamamide derivatives in the treatment of asthma, allergic diseases, hay fever, skin rashes, inflammatory bowel diseases, arthritis, adult respiratory distress syndrome (ARDS), migraine, cardiac shock, septic shock, thrombosis, hypotension, hypertension and ischemia.

BACKGROUND OF THE INVENTION

Tyrphostins are a group of low molecular weight compounds, having the nucleus of benzylidenemalononitrile and/or hydroxycinnamamide. These compounds are reported to be inhibitors of protein tyrosine kinases and their use in the treatment of cancer has been recommended. I have found that these agents also inhibit the action of the mediators of asthma, inflammation and cardiovascular diseases.

Compounds having the nucleus of benzylidene malononitrile such as N-(2-(2,5-dihydroxyphenyl) ethenyl) formamide, identified with the trade-mark Erbstatin, have been known for several years. Such compounds have the following formula:



These compounds were initially described as inhibitors of protein tyrosine kinase and their use in cancer chemotherapy was recommended due to their effects on blocking the epidermal growth factor receptor (EGF) kinase (Umezawa, H. et al., J. Antibiotics, vol. 39, 170-173, 1986 and Yaish, P. et al., Science, vol. 242, 933-935, 1988).

U.S. Patent No. 4,686,308, granted August 11, 1987, protects a novel compound 2,2-formamidoethenyl-1,4-hydroxyquinone. Preparation comprises cultivation of a Streptomyces strain or by its mutant treated by ultraviolet irradiation, or by recombinant DNA techniques of gene coding. This compound purportedly has antitumour and antimicrobial activities, with inhibitory activity against tyrosine specific protein kinase.

Sseveral articles disclose tyrosine kinase inhibitors. One is entitled "Blocking of EGF-Dependent Cell Proliferation by EGF Receptor Kinase Inhibitors", Pnina Yaish, Aviv Gazit, Chaim Gilon, Alexander Levitzki, Science, vol.242, Nov. 11, 1988, pp. 933-935. This article describes the synthesis of a systematic series of low molecular weight protein tyrosine kinase inhibitors. They had progressively increasing affinity over a 2500-fold range toward the substrate site of epidermal growth factor (EGF) receptor kinase domain. These compounds inhibited EGF receptor kinase activity up to three orders of magnitude more than they inhibited insulin receptor kinase, and they also effectively inhibited the EGF-dependent autophosphorylation of the receptor. The most potent compounds effectively inhibited the EGF-dependent proliferation of A431/clone 15 cells with little or no effect on the EGF-independent proliferation of these cells. The potential use of tyrosine protein kinase inhibitors as antiproliferative agents is demonstrated.

Another is entitled "Tyrphostins Inhibit Epidermal Growth Factor (EGF)-Receptor Tyrosine Kinase Activity in Living Cells and EGF-stimulated Cell Proliferation", R. M. Lyall, A. Zilberstein, A. Gazit, C. Gilon, A. Levitzki and J. Schlessinger,

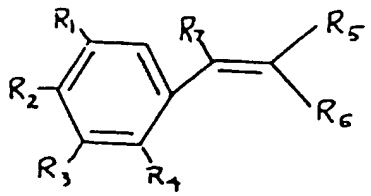
order: EGF receptor > p70gag-actin-v-fgr > pp60c-src > p130gag-v-fps, pp60v-src, with 50% inhibitory concentration values of 1.1, 4.2, 18, 70, and 87 μM, respectively. The phosphorylation of the tyrosine residues in particulate fractions from RR1022 cells expressing pp60v-src was inhibited by ST 638 in a dose-dependent way, while it had a negligible effect on the phosphorylations of threonine and serine residues. Kinetic analysis showed that ST 638 competitively inhibited the phosphorylation of an exogenous substrate by the EGF receptor kinase with a K_i of 2.1 μM. ST 638 noncompetitively inhibited autophosphorylation by EGF receptor kinase. These results indicate that ST 638 is a potent and specific inhibitor of tyrosine kinases in vitro, and that its inhibitory activity is caused by competing with the substrate protein for the tyrosine kinase binding site.

A fourth article relates specifically to Erbstatin: "Effective Synthesis of Erbstatin and its Analogs", E. L. Dulaney and C.A. Jacobsen, The Journal of Antibiotics, Vol. XL, No. 8, Aug. 1987, pp. 1207-1212. Erbstatin, purported to be a new potent inhibitor for tyrosine protein kinase (TPK), was isolated from the broth of Streptomyces sp. (MH435-hF3) and the structure was determined by X-ray crystallographic analysis. Erbstatin (3a) and its analogs were expected to be useful for the studies of the functions of oncogenes (tumour inducing genes), and may have therapeutic activity for the treatment of cancer.

SUMMARY OF THE INVENTION

The invention is directed to a composition useful in the treatment of inflammation induced diseases comprising:

(a) a compound of the formula



e Journal of Biological Chemistry, Vol. 264, No. 24, Aug. 25, 1989, pp. 14503-14509. Synthetic compounds called tyrphostins were examined for their effects on cells which are mitogenically responsive to epidermal growth factor (EGF). The writers studied in detail the effects of two tyrphostins on EGF binding, tyrosine phosphorylation in intact cells, EGF-receptor internalization, and mitogenesis. These compounds inhibited EGF-stimulated [^{3}H] thymidine incorporation in a specific manner and the degree of selectivity varied. Both compounds inhibited EGF-stimulated receptor autophosphyorylation and tyrosine phosphorylation of endogenous substrates in intact cells at doses that correlated with the IC_{50} for [^{3}H] thymidine incorporation. These results are consistent with the notion that tyrosine phosphorylation is a crucial signal in transduction of the mitogenic message delivered by EGF. The compound RG50864 demonstrated specificity at inhibiting EGF-stimulated cell growth compared with stimulation with either platelet-derived growth factor or serum. These novel synthetic inhibitors, specific for EGF-receptor kinase, allegedly offer a new method to inhibit EGF-stimulated cell proliferation which may be useful in treating specific pathological conditions involving cellular proliferation, including different types of cancers.

A third article is entitled "Specific Inhibitors of Tyrosine-Specific Protein Kinases: Properties of 4-Hydroxycinnamamide Derivatives in Vitro", T. Shiraishi, M. K. Owada, M. Tatsuka, T. Yamashita, K. Watanabe and T. Kakunaga, Cancer Research 49, 2374-2378, May 1, 1989. Inhibition by seven synthetic 4-hydroxycinnamamide derivatives, ST 271, ST 280, ST 458, ST 494, ST 633, ST 638 and ST 642, of tyrosine-specific protein kinases (tyrosine kinase) of oncogene or proto-oncogene products (p130gag-v-fps, p70gag-actin-v-fgr, pp60v-src, pp60c-src) and epidermal growth factor (EGF) receptor kinase were investigated. ST 638 (α-cyano-3-ethoxy-4-hydroxy-5-phenylthi- methylcinnamamide) strongly inhibited more of the tyrosine kinases than any of the other compounds. The susceptibilities of these tyrosine kinases to ST 638 increased in the following

wherein R_1 is H, OH, OCH_3, ETO;

R_2 is EtO, $CHC(CH_3)_2$, iso-Proline, CH_3SCH_2, H, OH, NO_2, OCH_3, OCH, R_6Cl;

R_3 is H, OH, OCH_3, phenyl SCH_2, $CH.(CH_3)_2$, iso-Proline, CH_3SCH_2;

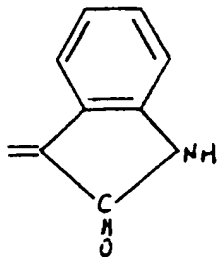
R_4 is H, OH;

R_5 is H, CN, COOH, NHCHO;

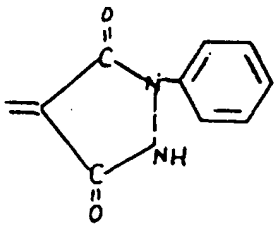
R_6 is H, CN, COOH, NHCHO, O, S;

R_7 is H, OH; and wherein

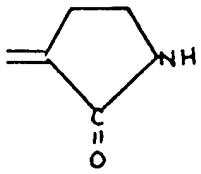
R_5 and R_6 can form the following cyclic structures:



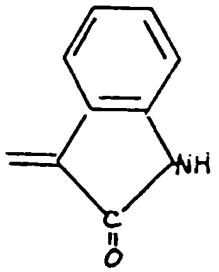
when R_1 and R_3 are CH_3SCH_2, R_2 is OH, and R_4 and R_7 are H;



when R_1 is ETO, R_2 is OH, R_3 is $PHSCH_2$, R_4 and R_7 are H;



.nen R_1 is ETO, R_2 is OH, R_3 is PhenylSCH$_2$ and R_4 and R_7 are H; and

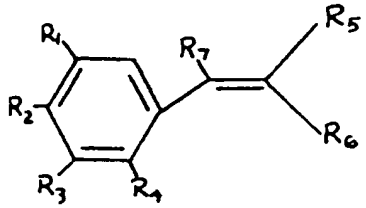


when R_1 and R_3 are iso-Proline, R_2 is OH, and R_4 and R_7 are H, and pharmaceutically acceptable acid addition salts thereof; and

(b) a pharmaceutically acceptable carrier.

The invention includes a composition for treating inflammatory diseases comprising:

(a) a benzylidene malononitrile of the formula:



wherein:

(1) R_1=OH, R_2=H, R_3=H, R_4=OH, R_5=NHCHO, R_6=H
(2) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CO_2H, R_6=H
(3) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CO_2H, R_6=CO_2H
(4) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=CN
(5) R_1=OH, R_2=OH, R_3=H, R_4=H, R_5=CO_2H, R_6=N
(6) R_1=OH, R_2=H, R_3=H, R_4=OH, R_5=H, R_6=NHCHO
(7) R_1=H, R_2=H, R_3=OH, R_4=H, R_5=CN, R_6=CN
(8) R_1=OH, R_2=H, R_3=H, R_4=OH, R_5=CN, R_6=CO_2H
(9) R_1=H, R_2=OH, R_3=OH, R_4=H, R_5=CO_2H, R_6=CN
(10) R_1=H, R_2=OH, R_3=OH, R_4=H, R_5=CN, R_6=CN
(11) R_1=OCH_3, R_2=OH, R_3=OH, R_4=H, R_5=CN, R_6=CN

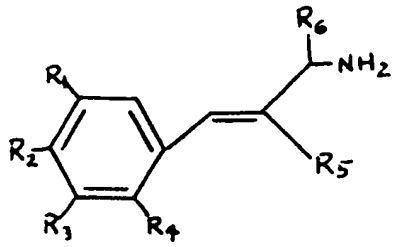
(12) R_1=OH, R^2=OH, R_3=OH, R_4=H, R_5=CN, R_6=CN
(13) R_1=OH, R_2=OH, R_3=OH, R_4=OH, R_5=CN, R_6=CN
(14) R_1=OH, R_2=OH, R_3=OH, R_4=H, R_5=NHCHO, R_6=H
(15) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=CN
(16) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=H
(17) R_1=OH, R_2=O_2N, R_3=H, R_4=H, R_5=CN, R_6=CN
(18) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=CN, R_7=OH
(19) R_1=CH_3O, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=CN
(20) R_1=OH, R_2=H, R_3=OH, R_4=H, R_5=CN, R_6=CN
(21) R_1=OH, R_2=OH, R_3=OH, R_4=H, R_5=CN, R_6=CN, R_7=OH
(22) R_1=H, R_2=CH_3O, R_3=H, R_4=H, R_5=CO_2H, R_6=CN
(23) R_1=H, R_2=F_1Cl, R_3=H, R_4=H, R_5=CO_2H, R_6=CN
(24) R_1=CH_3O, R_2=OH, R_3=CH_3O, R_4=H, R_5=CO_2H, R_6=CN
(25) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CO_2H, R_6=CN
(26) R_1=H, R_2=OCH, R_3=H, R_4=H, R_5=CO_2H, R_6=CN
(27) R_1=OH, R_2=H, R_3=H, R_4=H, R_5=CN, R_6=CO_2H

and pharmaceutically acceptable acid addition salts thereof; and

(b) a pharmaceutically acceptable carrier.

The invention also includes a composition for treating inflammatory diseases comprising:

(a) a cinnamamide of the formula:



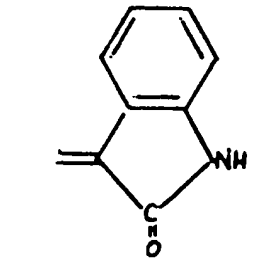
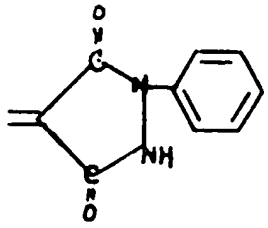
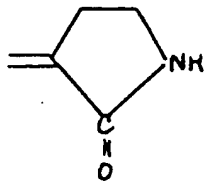
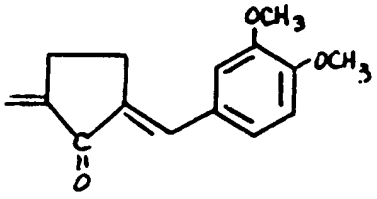
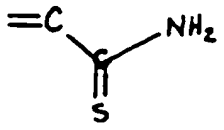
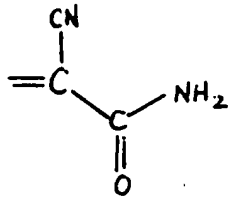
wherein:

(1) R_1=ETO, R_2=OH, R_3=PhenylSCH$_2$, R_4=H, R_5=CN, R_6=O
(2) R_1=CH.CMe_2, R_2=OH, R_3=CH.Me_2, R_4=H, R_5=CN, R_6=O
(3) R_1=ETO, R_2=OH, R_3=PhenylSCH$_2$, R_4=H, R_5=CN, R_6=S
(4) R_1=OH, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=S
(5) R_1=iso-Proline, R_2=OH, R_3=iso-Proline, R_4=H, R_5=CN, R_6=O

(6) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=O

(7) R_1=OH, R_2=OH, R_3=OH, R_4=H, R_5=CN, R_6=O; or

(8) R_1=OH, R_2=OH, R_3=OH, R_4=I, R_5=CN, R_6=S; or wherein R_5 and R_6 can combine to one of the following structures; and having R_1=OCH_3, iso-proline or OH, R_2=OCH_3 or OH, R_3=iso-proline or OCH_3

and acid addition salts thereof; and

(b) a pharmaceutically acceptable carrier.

In the composition as illustated above, R_1 can be OH and R_2 and R_3 can be H, R_4 can be OH, R_5 can be NHCHO and R_6 and R_7 can be H. In the composition, the carrier is distilled water. In the composition as described, compound (a) can be present in compound (b) at a concentration ranging from 0.5 mg/l to 100 mg/l. The composition can include an effective amount of an iron chelation agent, and/or oxygen radicals scavenger.

The composition is administered orally, as an aerosol, subcutaneously or intravenously. The composition can be in the form of a tablet. The composition can be used in the treatment of asthma, allergic diseases, hay fever, skin rashes, inflammatory bowel diseases, arthritis, adult respiratory distress

syndrome (ARDS), migraine, cardiac shock, septic shock, thrombosis, hypotension, hypertension and ischemia.

DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

Platelet activating factor (PAF) and Leukotriene D_4 (LTD_4) are important components of bronchial hyperresponsiveness and inflammation. Elucidation of their mode of cellular action is a crucial step towards the understanding of pathophysiological states caused by PAF and LTD_4 and subsequently the management of diseases associated with those molecules.

Initially, the role of protein-tyrosine phosphorylation in the signal transduction of PAF was investigated in rabbit platelets. Two tyrosine kinase inhibitors, N-(2-(2,5-dihydroxyphenyl)ethenyl)formamide-methanolate (coded; TR-1A) and α-cyano-3,4-dihydroxythiocinnamamide (TR-18) were found to inhibit PAF responses at the IC_{50} = 15 and 50 μg/ml, respectively. Inhibition of protein-tyrosine phosphorylation blocked PAF-induced phosphoinositide breakdown, membranous protein kinase C activity, platelet aggregation and serotonin release. These data imply that protein-tyrosine phosporylation plays a critical role in PAF-signal transduction systems. This suggests that the PAF receptor may be a tyrosine kinase or a tyrosine phosphorylatable protein coupled to a phospholipase C, or alternatively coupled to a phospholipase C amenable to phosphorylation by tyrosine kinases.

This work provides firm evidence that specific inhibitors of protein-tyrosine kinase should prove to be valuable tools in the management of bronchial hyperresponsiveness and inflammation.

The drug TR-1A was initially believed to be useful for the study of the functions of oncogenes (tumour inducing genes) due to its action on the inhibition of protein tyrosine kinase.

due to the fact that the receptors for a number of mediators of inflammatory and allergic reactions might have protein tyrosine kinase activity, I have discovered that the drug TR-1A can inhibit the activation of these mediators' receptors and affect the normal interaction of these receptors with their effector system.

Existing drugs used in the treatment of asthma, inflammatory diseases, or allergic diseases, are designed either to inhibit certain enzymes responsible for the syntheses of these mediators, or to block their mode of signal transduction. TR-1A, by inhibiting protein tyrosine kinase, a common enzyme for the transduction of signals for many mediators, should be more effective in preventing the above diseases, since it will work against many mediators at the same time.

I have found the drug TR-1A inhibits platelet activating factor (PAF) (an inflammatory mediator) responses. These included aggregation of platelets and release of serotonin. TR-1A also inhibited constriction of guinea pig's trachea in response to leukotiene D_4 (LTD_4) (a major mediator of allergic and asthmatic reactions). From this, it is evident that TR-1A has important use in the treatment of asthma, allergy cardiovascular and inflammatory diseases.

From the foregoing demonstrations, it follows that the compound TR-1A is useful for the treatment of virtually all kinds of inflammatory diseases (eg. arthritis, inflammatory bowel disease, etc.), all kinds of allergic diseases (eg. asthma, rhenitis, skin allergy, hay fever, systemic anaphylaxis), and all kinds of heart and vascular diseases (eg. septic shock, ARDS, cardiogenic shock, arrythmias, hypotension, hypertension, thrombosis and blood clot).

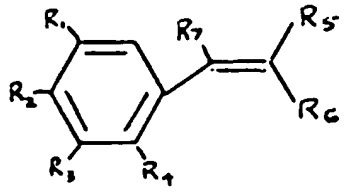
I have discovered that the compounds of the invention block the action of PAF and LTD_4. Although the receptors for these molecules are not characterized, nevertheless, it is

believed that these agents inhibit tyrosine phosphorylation of the receptor. Alternatively, the compounds of the invention are interfering with biochemical parameters involved in the transduction of signals for PAF or LTD_4. Such biochemical parameters could be the component of guanyl nucleotide regulatory proteins (G-proteins), phospholipases, protein kinases or phosphatases.

The compounds of the invention, with their novel uses, can be broadly divided into two main specific groups:

Group A Compounds (Benzylidene Malononitrile Compounds)

A composition for treating asthma, allergic diseases, hay fever, skin rashes, arthritis, inflammatory diseases (bowel, colon, etc.), adult respiratory distress syndrome (ARDS), septic shock, cardiac shock, thrombosis, hypertension, hypotension, tissue ischemia and migraine, comprising a benzylidene malononitrile of the formula:



wherein:

(1) R_1=OH, R_2=H, R_3=H, R_4=OH, R_5=NHCHO, R_6=H (TR-1A)
(2) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CO_2H, R_6=H
(3) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CO_2H, R_6=CO_2H
(4) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=CN
(5) R_1=OH, R_2=OH, R_3=H, R_4=H, R_5=CO_2H, R_6=N
(6) R_1=OH, R_2=H, R_3=H, R_4=H, R_5=H, R_6=NHCHO
(7) R_1=H, R_2=H, R_3=OH, R_4=H, R_5=CN, R_6=CN
(8) R_1=OH, R_2=H, R_3=H, R_4=OH, R_5=CN, R_6=CO_2H
(9) R_1=H, R_2=OH, R_3=OH, R_4=H, R_5=CO_2H, R_6=CN

(10) R_1=H, R_2=OH, R_3=OH, R_4=H, R_5=CN, R_6=CN

(11) R_1=OCH_3, R_2=OH, R_3=OH, R_4=H, R_5=CN, R_6=CN

(12) R_1=OH, R^2=OH, R_3=OH, R_4=H, R_5=CN, R_6=CN

(13) R_1=OH, R_2=OH, R_3=OH, R_4=OH, R_5=CN, R_6=CN

(14) R_1=OH, R_2=OH, R_3=OH, R_4=H, R_5=NHCHO, R_6=H

(15) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=CN

(16) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=H

(17) R_1=OH, R_2=O_2N, R_3=H, R_4=H, R_5=CN, R_6=CN

(18) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=CN, R_7=OH

(19) R_1=CH_3O, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=CN

(20) R_1=OH, R_2=H, R_3=CH, R_4=H, R_5=CN, R_6=CN

(21) R_1=OH, R_2=OH, R_3=OH, R_4=H, R_5=CN, R_6=CN, R_7=OH

(22) R_1=H, R_2=CH_3O, R_3=H, R_4=H, R_5=CO_2H, R_6=CN

(23) R_1=H, R_2=FICl, R_3=H, R_4=H, R_5=CO_2H, R_6=CN

(24) R_1=CH_3O, R_2=OH, R_3=CH_3O, R_4=H, R_5=CO_2H, R_6=CN

(25) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CO_2H, R_6=CN

(26) R_1=H, R_2=OCH, R_3=H, R_4=H, R_5=CO_2H, R_6=CN

(27) R_1=OH, R_2=H, R_3=H, R_4=H, R_5=CN, R_6=CO_2H

Results of Experiments Conducted with Group A Compounds

Initial Experiments

A number of initial experiments were performed with N-(2-(2-,5-dihydroxyphenyl)ethenyl) formamide methanolate (TR-1A), to inhibit biological activity of PAF on rabbit platelets.

Rabbit platelets were prepared according to the well-known method of Pinckard et al. J. Immunol. 123, 1847-1853, 1979. Platelets at 2 x 10^8/ml were challenged with PAF (0.2 nM) at 37° in Tyrode's buffer, pH 7.2. Platelets (0.5 ml) were tested in a Bio-Data aggregometer for aggregation in response to PAF. TR-1A or PAF was added in 50 ul of 0.25% BSA in Tyrode's buffer. Activities were measured as percent increase in light transmission.

As can be seen from Table 1, TR-1A, at 10 μg/ml inhibited PAF-induced platelets aggregation by approximately 30%. At 25 μg/ml, it is seen that TR-1A blocked the action of PAF by 100%.

Table 1

Effect of Various Concentrations of TR-1A on PAF Induced Rabbit Platelets Aggregation

TR-1A Conc. (μg/ml)	PAF (200 PM) Induced Aggregation (% of Control mean of five experiments
0	100
5	100
10	30
25	0
50	0

Second Set of Experiments

In another set of studies, at similar concentrations, it was found that TR-1A inhibited PAF induced serotonin release from rabbit platelets. Platelets were labelled with [^{3}H] serotonin for 1 hour. After this period of time, the unincorporated [^{3}H] serotonin was washed off and the platelets were used for challenge with PAF in the presence and absence of TR-1A. TR-1A, from 10 μg/ml, was found to inhibit PAF (200 PM) induced serotonin release. The IC_{50} was determined to be about 10 μg/ml. At 25 μg/ml, it was noted that TR-1A entirely blocked PAF induced serotonin release.

TR-1A, at concentrations below 10 μg/ml, did not appreciably prevent PAF induced serotonin release from rabbit platelets. At 20 μg/ml, TR-1A inhibited PAF action by about 50%. At 25 μg/ml, TR-1A almost completely blocked the action of PAF. These determinations are tabulated in Table 2.

Table 2

Inhibitory Effect of Various Concentrations of TR-1A on PAF Induced Serotonin Release from Rabbit Platelets

TR-1A Conc. (μg/ml)	Serotonin Release (% of Control) mean of five experiments
0	100
0.5	97
1	102
2	98
5	92
10	42
20	0
25	0
50	0

Example

In order to evaluate the specific site of action of TR-1A on platelets, a series of experiments were performed to investigate the cellular second messenger system following PAF activation. Platelets were labelled with [^{3}H] inositol which became incorporated into phosphatidylinositol. The metabolism of phosphatidylinositol in response to PAF was then investigated. the results are tabulated in Table 3.

As Table 3 shows, PAF (200 PM), without the presence of TR-1A, caused rapid hydrolysis of phosphatidylinositide. However, when the platelets were first pretreated with various concentrations of TR-1A for 5 minutes prior to the addition of PAF, the formation of metabolites of phosphoinositide (inositol monophosphate, inositol bisphosphate and inositol trisphosphate) were inhibited. The IC_{50} for TR-1A was found to be between 20 and 25 μg/ml. At 50 μg/ml, it was noted that TR-1A entirely blocked the action of PAF. Similarly, TR-1A blocked the

activation of protein kinase C induced by PAF. Protein kinase (PKC) is also a major component of a cell signalling system.

In this assay system, platelets were preincubated for 5 minutes with TR-1A (0-50 µg/ml) and subsequently treated with PAF (200 PM) for 1 minute. The sample material was chromatographed on a mono Q column. 0.5 ml of NP 40-solubilized particulate protein from the platelet extracts was chromatographed, and the column fractions assayed for phosphorylating activity. It was found that TR-1A from 10 µg/ml inhibited (>40%) the activation of PKC by PAF. At 25 µg/ml, TR-1A blocked about 80% of the action of PAF. These results suggest that TR-1A blocks PAF induced activation of rabbit platelets. Thus it follows that TR-1A can be used in the treatment of diseases where PAF plays an important role (such as asthma, cardiovascular and inflammatory diseases).

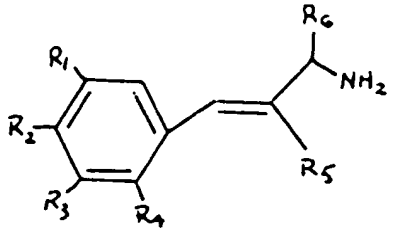
Table 3

Inhibitory Effects of TR-1A on PAf Induced Polyphosphoinositide Metabolism in Rabbit Platelets

TR-1A (µg/ml)	Phosphoinosidide Metabolites Formation (% of control)		
	IP1	IP2	IP3
0	100	100	100
0.5	100	100	100
1	100	100	100
5	80	90	100
10	65	70	90
25	50	40	35
50	30	15	10

Group B Compounds (Hydroxy Cinnamamide Compounds)

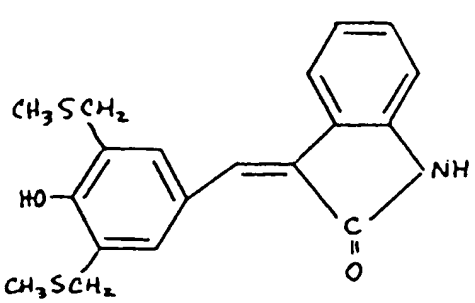
Composition containing hydroxy cinnamamide compounds according to the following formula are useful for treating asthma, allergic diseases, hay fever, skin rashes, arthritis, inflammatory diseases (bowel, colon, etc.), ARDS, septic shock, cardiac shock, thrombosis, hypertension, hypotension, tissue ischemia and migraine. The hydroxycinnamamides have the following formula:

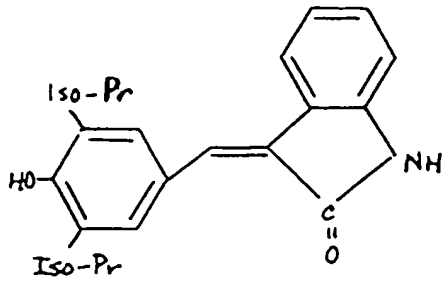


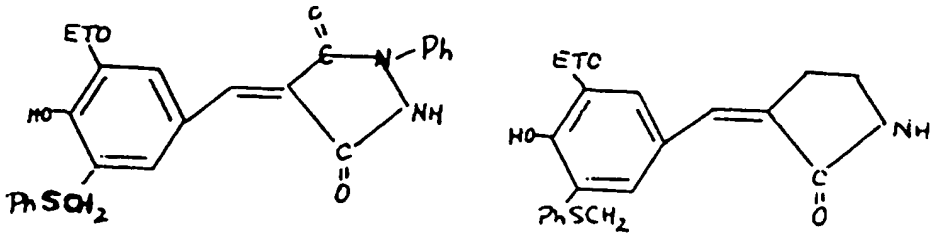
wherein:

(1) R_1=ETO, R_2=OH, R_3=PhenylSCH$_2$, R_4=H, R_5=CN, R_6=O
(2) R_1=CH.CMe$_2$, R_2=OH, R_3=CH.CMe$_2$, R_4=H, R_5=CN, R_6=O
(3) R_1=ETO, R_2=OH, R_3=PhenylSCH$_2$, R_4=H, R_5=CN, R_6=S
(4) R_1=OH, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=S
(5) R_1=iso-Proline, R_2=OH, R_3=iso-Proline, R_4=H, R_5=CN, R_6=O
(6) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=O
(7) R_1=OH, R_2=OH, R_3=OH, R_4=H, R_5=CN, R_6=O
(8) R_1=OH, R_2=OH, R_3=OH, R_4=I,F,Cl, R_5=CN, R_6=S

The hydroxycinnamamide can also have the following structures:







Experiments Conducted with Group B Compounds

Results of the studies conducted with four compounds of the groups B, α-cyano-4-hydroxy-3, 5-diisopropylcinnamamide and α-cyano-3, 4-dihydroxythiocinnamamide are shown in Table 4 with LTD_4 induced smooth muscle contraction. Leukotriene D_4 is the major component of bronchial asthma, contracts bronchial and tracheal smooth muscle cells at the concentrations of $\geq 10^{-10}$M. The effects of four different protein tyrosine kinase inhibitors were evaluated against contraction induced by LTD_4 in isolated guinea pig tracheal preparation. Trachea from guinea pigs were suspended in a jacketed organ bath containing oxygenated kreb's-Henseleit colution. The tissues were allowed to equilibrate for 60-90 minutes under 1.5 g tension and then optimal tension obtained using electrical field stimulation at 0.25 g tension increments. Isometric force generation was measured with Grass FT 0.3 force transducer, and recorded on a polygraph. Responses of each tissue to Acetylcholine (10^{-3}M) was first evaluated. The tissues were then washed and stimulated with various concentrations of LTD_4 (ranging from 10^{-10}M to 3×10^{-7}M).

As can be seen from Table 4, pretreatment of tissue with tyrosine kinase inhibitors (10 μg/ml) for 60 minutes, caused significant inhibition of LTD_4 (3×10^{-7}) induced smooth muscle contraction. The most potent of all the four tested inhibitors was found to be α-cyano-4-hydroxy-3, 5-diisopropylcinnamamide. This was greater than α-cyano-3, 4-dihydroxythiocinnamamide which was greater than 2,5-bis-(3,4-dimethoxybenzylidene) cyclopentanone which was more effective than N-(2-(2,5-dihydroxyphenyl)ethenyl) formamide methanolate.

Table 4

Effects of Tyrosine Kinase Inhibitors on Leukotriene D_4 (3×10^{-7}M) Induced Guinea Pig Tracheal Contraction

Inhibitors (10 µg/ml)	% Inhibition of LTD_4 Induced Smooth Muscle Contraction mean of 3 experiments
α-cyano-4-hydroxy-3,5-diisopropylcinnamamide	56.5
α-cyano-3,4-dihydroxythiocinnamamide	54.3
2,5-bis(3,4-dimethoxy benzylidine) cyclopentanone	50.4
N-(2,(2,5-dihydroxyphenyl)ethenyl)formamide methanolate	31.7

These results clearly indicate that tyrosine kinase inhibitors with the common structure of hydroxycinnamamide are effective in preventing leukotriene D_4 and presumably other arachidonic acid metabolites which induce smooth muscle contraction. Therefore, these substances can be used in the treatment of asthma and other inflammatory and allergic diseases, in which arachidonic acid metabolites play a major role.

Conclusion

Leukotrienes are polyunsaturated conjugated trienes derived from arachidonic acid. They consist of leukotriene A_4 (LTA_4), LTB_4, LTC_4, LTD_4, and LTE_4. Other arachidonic acid metabolites are called prostaglandins and thromboxane A_2. Their role in inflammatory diseases such as arthritis and in airway asthma has been recognized for several years. For example, LTC_4, LTD_4, LTE_4, PGD_2, and TXA_2 are potent smooth muscle constrictors. They also contract vascular smooth muscles. LTB_4, PAF and PGE_2 are major components of inflammatory diseases such as arthritis. Recruiting leukocyte to the site of inflammation causes these cells to degranulate and release their dysosomal enzyme. The combination of intracellular enzymes and leukocytes accumulation causes acute inflammation. Other arachidonic acid metabolites are shown to be involved in the diseases associated with cardiovascular system. For example, TXA_2 which is a potent constrictor of vasculature, can induce hypertension. To the contrary, PGI_2, a potent vasodilator, induces hypotension. Cardiac shock and ischemia are also associated with PGI_2. In regard to septic shock and ARDS, PAF may play a major role. Several available reports indicate that PAF, to a large extent, and TXA_2, to a lesser extent, play a critical role in the initiation of cardiac shocks associated with endotoxin (septic shock). In regard to migraine, several prostaglandins such as PGE_2, E_1, etc., have been shown to have an important role in the development of migraine. It follows, therefore, that the tyrosine kinase inhibitors of the invention could be valuable drugs for inhibition of the cellular activation by a number of

prostaglandins, leukotrienes, and PAF and thus can be valuable tools for the treatment of the above diseases.

Mode of Application

The compounds of Groups A or B may be present in the composition of the invention at a concentration in the range of 0.5-100 mg/l or kg of body weight of a pharmaceutically acceptable carrier.

Suitable doses are 1-1,000 mg/kg, especially 1-10 mg, preferably taken 2 to 3 times daily, orally, subcutaneously, intravenously or by aerosol. The pharmaceutically acceptable carrier may be distilled water, a mixture of saline, glucose, lactose or ethylcellulose N100 and water or starch talc. The composition of the invention may be administered orally, by aerosol, subcutaneously or intravenously. Tablets for oral ingestion may be made via compression of approximately 100 mg of a compound of Group A or B, 100 mg of an iron chelator, 200 mg of lactose and 100 mg Avicel.

Capsules may be prepared by making micelles of liposomal drugs with lecithin. Micelle injections can be made either in water and propylene glycol with an upwardly adjusted pH in phosphate buffer. The product is typically sterilized through a filter. The micelle can be made in 20 percent propylene glycol and a preservative such as ascorbic acid. The aerosol composition can be made by making liposomes of the compounds of Group A or B in a pharmaceutically acceptable buffer/lecithin, with preservative, and solubilizing agent such as 0.1% ethanol.

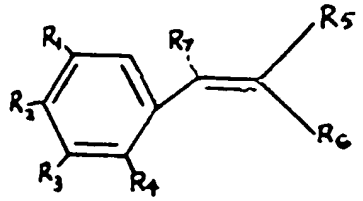
As will be apparent to those skilled in the art in the light of the foregoing disclosure, many alterations and modifications are possible in the practice of this invention without departing from the spirit or scope thereof. Accordingly, the scope of the invention is to be construed in accordance with the

Substance defined by the following claims.

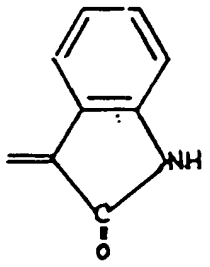
HE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A composition useful in the treatment of inflammation induced diseases comprising:

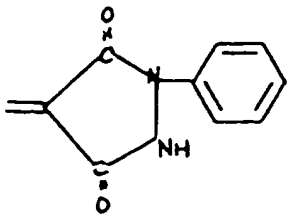
(a) a compound of the formula



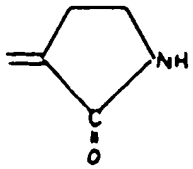
wherein R_1 is H, OH, OCH_3, ETO;
R_2 is EtO, $CHC(CH_3)_2$, iso-Proline or a halogen, CH_3SCH_2, H, OH, NO_2, OCH_3, OCH, halogen, R_6Cl;
R_3 is H, OH, OCH_3, phenyl SCH_2, $CH.(CH_3)_2$, iso-Proline, CH_3SCH_2 or halogen;
R_4 is H, OH;
R_5 is H, CN, COOH, NHCHO;
R_6 is H, CN, COOH, NHCHO, O, S;
R_7 is H, OH; and wherein
R_5 and R_6 can form the following cyclic structures:



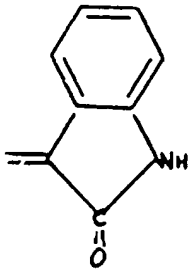
when R_1 and R_3 are CH_3SCH_2, R_2 is OH, and R_4 and R_7 are H;



when R_1 is ETO, R_2 is OH, R_3 is $PhSCH_2$, R_4 and R_7 are H;



when R_1 is ETO, R_2 is OH, R_3 is PhenylSCH_2 and R_4 and R_7 are H; and

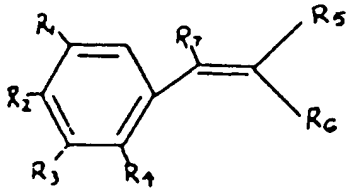


when R_1 and R_3 are iso-Proline, R_2 is OH, and R_4 and R_7 are H, and pharmaceutically acceptable acid addition salts thereof; and

(b) a pharmaceutically acceptable carrier.

2. A composition for treating inflammatory diseases comprising:

(a) a benzylidene malononitrile of the formula:



wherein:

(1) R_1=OH, R_2=H, R_3=H, R_4=OH, R_5=NHCHO, R_6=H

(2) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CO_2H, R_6=H

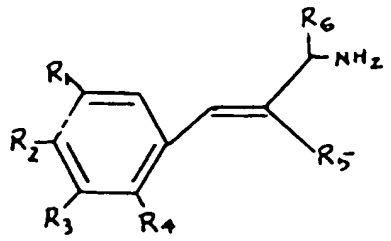
(3) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CO_2H, R_6=CO_2H
(4) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=CN
(5) R_1=OH, R_2=OH, R_3=H, R_4=H, R_5=CO_2H, R_6=N
(6) R_1=OH, R_2=H, R_3=H, R_4=OH, R_5=H, R_6=NHCHO
(7) R_1=H, R_2=H, R_3=OH, R_4=H, R_5=CN, R_6=CN
(8) R_1=OH, R_2=H, R_3=H, R_4=OH, R_5=CN, R_6=CO_2H
(9) R_1=H, R_2=OH, R_3=OH, R_4=H, R_5=CO_2H, R_6=CN
(10) R_1=H, R_2=OH, R_3=OH, R_4=H, R_5=CN, R_6=CN
(11) R_1=OCH_3, R_2=OH, R_3=OH, R_4=H, R_5=CN, R_6=CN
(12) R_1=OH, R^2=OH, R_3=OH, R_4=H, R_5=CN, R_6=CN
(13) R_1=OH, R_2=OH, R_3=OH, R_4=OH, R_5=CN, R_6=CN
(14) R_1=OH, R_2=OH, R_3=OH, R_4=H, R_5=NHCHO, R_6=H
(15) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=CN
(16) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=H
(17) R_1=OH, R_2=O_2N, R_3=H, R_4=H, R_5=CN, R_6=CN
(18) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=CN, R_7=OH
(19) R_1=CH_3O, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=CN
(20) R_1=OH, R_2=H, R_3=OH, R_4=H, R_5=CN, R_6=CN
(21) R_1=OH, R_2=OH, R_3=OH, R_4=H, R_5=CN, R_6=CN, R_7=OH
(22) R_1=H, R_2=CH_3O, R_3=H, R_4=H, R_5=CO_2H, R_6=CN
(23) R_1=H, R_2=F_1Cl, R_3=H, R_4=H, R_5=CO_2H, R_6=CN
(24) R_1=CH_3O, R_2=OH, R_3=CH_3O, R_4=H, R_5=CO_2H, R_6=CN
(25) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CO_2H, R_6=CN
(26) R_1=H, R_2=OCH, R_3=H, R_4=H, R_5=CO_2H, R_6=CN
(27) R_1=OH, R_2=H, R_3=H, R_4=H, R_5=CN, R_6=CO_2H

and pharmaceutically acceptable acid addition salts thereof; and

(b) a pharmaceutically acceptable carrier.

3. A composition for treating inflammatory diseases comprising:

(a) a cinnamamide of the formula:



wherein:

(1) R_1=ETO, R_2=OH, R_3=PhenylSCH$_2$, R_4=H, R_5=CN, R_6=O

(2) R_1=CH.CMe$_2$, R_2=OH, R_3=CH.CMe$_2$, R_4=H, R_5=CN, R_6=O

(3) R_1=ETO, R_2=OH, R_3=PhenylSCH$_2$, R_4=H, R_5=CN, R_6=S

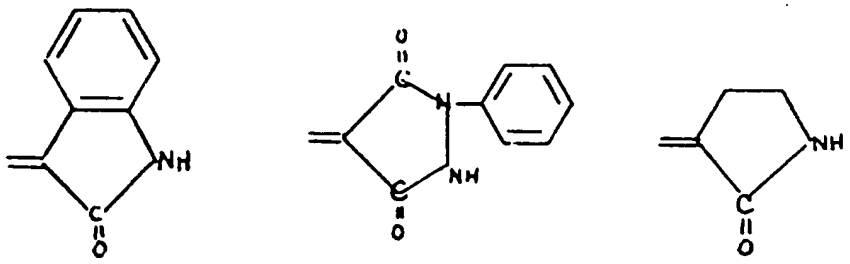
(4) R_1=OH, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=S

(5) R_1=iso-Proline, R_2=OH, R_3=iso-Proline, R_4=H, R_5=CN, R_6=O

(6) R_1=H, R_2=OH, R_3=H, R_4=H, R_5=CN, R_6=O

(7) R_1=OH, R_2=OH, R_3=OH, R_4=H, R_5=CN, R_6=O; or

(8) R_1=OH, R_2=OH, R_3=OH, R_4=I,F,Cl, R_5=CN, R_6=S; or wherein R_5 and R_6 can combine to one of the following structures:



and acid addition salts thereof; and

(b) a pharmaceutically acceptable carrier.

4. A composition as claimed in claim 1 wherein R_1 is OH, R_2 and R_3 are H, R_4 is OH, R_5 is NHCHO and R_6 and R_7 are H.

5. A composition as claimed in claim 4 wherein the carrier is distilled water.

6. A composition as claimed in claim 1 wherein compound (a) is present in compound (b) at a concentration ranging from 0.5 mg/l to 100 mg/l.

7. A composition as claimed in claim 6 including an effective amount of an iron chelation agent.

8. A composition as claimed in claim 1, 2 or 3 wherein the composition is administered orally, as an aerosol, subcutaneously or intravenously.

9. A composition as claimed in claim 1, 2 or 3 wherein the composition is in the form of a tablet.

10. A composition as claimed in claim 1, 2 or 3 wherein the composition is used in the treatment of asthma, allergic diseases, hay fever, skin rashes, inflammatory bowel diseases, arthritis, adult respiratory distress syndrome (ARDS), migraine, cardiac shock, septic shcok, thrombosis, hypotension, hypertension and ischemia.

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